DNA nanotechnology as a tool to manipulate lipid bilayer membranes

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Summary

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Great efforts have been dedicated to use DNA as a building block in nanotechnology because of its versatile properties, such as high specificity and programmability to form complex structures. The rapid development of chemical methodology has been proven to be useful not only for the preparation of pristine DNA and RNA, but also to make base, or backbone modifications of the oligonucleotides as well as many analogues. The structural variety has been further expanded from 2D-patterns or 3D-nano-objects, which are basically fabricated with pristine DNA, to DNA hybrids, such as DNA-nanoparticle hybrids including inorganic or soft materials. Specially, the phosphoramidite chemistry has been adapted for the modification of DNA with hydrophobic polymers or lipids, yielding DNA amphiphiles. These DNA amphiphiles can be used to perform surface modifications of biological membranes.

In **Chapter 2**, we have established a new strategy for anchoring oligonucleotides in vesicle membranes enabled by attaching hydrophobic units to the nucleobase. The membrane anchors are incorporated into the oligonucleotide by automated solid phase synthesis allowing precise control over the position and number of hydrophobic units within a DNA sequence. A FRET system was used to prove the incorporation and stability of lipid-DNA on the liposomal membrane. Single-stranded DNA functionalized with four lipid-modified nucleobases was stably grafted onto the membrane of lipid vesicles for at least 24 hours.

After demonstrating the stability of lipid-DNA in the liposomal bilayer, lipid-DNA induced liposome fusion was studied in **Chapter 3**. The result demonstrates the importance of the DNA-anchoring strategy in hybridization-induced vesicle fusion, as not only the structural properties of the unit itself, but also the number of anchoring units determine the fusogenic properties. It was found that the orientation of DNA hybridization and the number of anchoring units played a crucial role in liposomal fusion. The zipper-orientated hybridization is more efficient than non-zipper-orientated hybridization, which supports that double-stranded DNA close to the vesicle surface could bring the docked vesicles in close proximity to enhance full fusion. In the zipper orientation, the hybridization event including vesicles with complementary sequences brings their membranes in close proximity than in the non-zipper configuration. Meanwhile, compared to vesicles functionalized with single-anchored or double-anchored DNA, liposomes containing quadruple-anchored oligonucleotides were proved to be highly fusogenic, achieving considerable full fusion of up to 29% without notable leakage, which might be related to the higher affinity of a quadruple lipid anchor to the membrane. With this fusion system the most efficient fusogenic DNA probes were produced known to date.

To further extend the functionality of DNA-based vesicles, the study presented in **Chapter 4** was focused on the functionalization of DNA amphiphiles in a phospholipid bilayer. We explored DNA hybridization and the dynamic exchange of DNA sequences on the surface of liposomes by simple addition of DNA sequences with two FRET systems. FRET between C594 (acceptor) and U4T-18-grafted Rh-DHPE-containing (donor) vesicles demonstrated that DNA hybridization was achieved on the surface of liposome. Subsequently, a 20-mer DNA oligonucleotide was introduced to replace U4T-18 from C594 due to full hybridization. The disassembly of C594 from the vesicle surface was realized since C594 was attached to the liposome employing 14 complementary nucleotides while the removal strand formed a duplex involving 20 nucleotides. The hybridization energy for the latter is larger than for a 14mer duplex structure. Afterwards, the free U4T-18 hybridized with C488, which is a donor for Rh-DHPE-containing vesicles. Moreover, a DNA based amplification process was

performed on the surface of liposomes with a DNA probe, M2-Cy3. A remarkable fluorescence intensity of Cy3 was obtained due to the DNA hybridization chain reaction, which confirmed the capability of the multiplication of surface functionalities from a single DNA anchoring unit on the vesicle surface.

At the end of the thesis, a more complex membrane system was functionalized with DNA. In **Chapter 5**, the incorporation of nucleobase quadruple-anchored DNA in the surface layer of zebrafish embryos was evaluated. The payloads connected by the supramolecular tether DNA can be reversibly removed employing a removal strand, which represents a very mild stimulus just requiring the addition of a DNA sequence not affecting the life of the fish. Similar as on the vesicle surface, we successfully demonstrated the performance of a DNA mediated amplification process on the fish skin. The hybridization chain reaction allows attachment of multiple moieties on a single anchored DNA strand allowing multiplication of cargoes or signals on the surface. Moreover, it was shown that surface modification of model membranes in form of liposomes by various DNA nanotechnology procedures could be easily transferred to the live animal. This allows establishment of DNA based surface functionalization procedures and their facile and fast implementation in zebrafish. Due to the broad application of zebrafish as animal model in drug development, toxicology and nanoparticles characterization in living systems, we believe the platform presented here allows amalgamation of DNA nanotechnology tools with live animals and enables efficient bio-barcoding as well as *in vivo* tracking.

Overall, this thesis has shown that chemical synthesis is a valuable tool to produce functional DNA molecules to increase the complexity of membrane engineering approaches. This was achieved by modification of nucleobases by hydrophobic units and solid phase synthesis to fabricate amphiphilic DNA strands. The piercing of hydrophobic units on the DNA into the inner part of phospholipid membranes leads to stable anchoring. With this as a starting point, the dynamic process of vesicle fusion was achieved, which might be important in the future for synthesizing minute amount of compounds by exploiting content mixing of liposomes filled with different

reactants. Similarly, the fusion approach might be utilized in the context of actively transporting vesicle payloads in cells. In this case, the DNA hybridization will fuel the fusion of vesicle with cell membranes to deliver cargoes in the cytosol without being dependent on endocytosis processes. One could even think of transferring this concept to live animals as demonstrated in this thesis for zebrafish. It is without any doubt that DNA amphiphiles bear lots of future potential for sophisticated DNA nanotechnology functions in the realm of synthesis biology.